CERTIFICATION THAT TRANSLATION IS TRUE AND ACCURATE

I, *Jing LING*, state that the English translation attached hereto is a true and accurate translation of the attached Chinese Patent Application No. *94102799.6*, filed on *March 15. 1994*.

Date: *February 20, 2008*

NGEDOCS: 1481228.1

凌婧

ENGLISH TRANSLATED VERSION OF ZL94102799.6

Lixinkang

Abstract

The present invention relates to a medicament called Lixinkang for the treatment of myocarditis disease. The said medicament is a growth-stimulating peptide of the myocardial cells (GMGSP), which was isolated from the hearts of healthy infant mammals such as infant pigs, infant cattle and so on. And it is a polypeptide mixture with the molecular weight 5000-12000 Da, and its biological activity is stable. The said medicament can stimulate DNA synthesis and protein synthesis of primarily cultured myocardial cells, and promote cellular cleavage and proliferation, and protect myocardial cell membrane. In clinic, it can be applied to take orally or muscle injection, or intravenous injection with other medicaments and it has made a notable impact on the treatment of myocardial disease.

Claims

1. A medicament for the treatment of myocarditis—a growth-stimulating peptide of the myocardial cells (GMGSP) isolated from the hearts of healthy infant mammals, wherein the said GMGSP has the followed characteristics:

(1) it is stable at pH 2-9;

(2) its biological activity did not change when the said GMGSP was heated at 95-100℃ for 10 minutes or at 60-70℃ for 30 minutes;

(3) but its biological activity was lost when being placed in proteolytic enzymes at 37℃ for two hours;

(4) a polymer was formed at 22℃-30℃ in aqueous solution, but its biological activity did not have obvious change;

(5) its biological activity did not change if the said GMGSP was lyophilized and sealed with 3%-8% mannitol and stored at room temperature for 1.5 years, or at 4℃ for 2 years, or at -20℃ for 3 years;

(6) HPLC analysis indicated that the said GMGSP is composed of four components; the relative peaks and retention times of each component were respectively 10.4% (2.88 minutes), 6.4% (3.93 minutes), 36.3% (5.09 minutes) and 7.3% (7.41 minutes), and each component has biological activity;

(7) the molecular weights of two bands displayed by SDS-PAGE analysis were respectively 8500 Da and 10800 Da, and the average molecular weight displayed by HPLC analysis was 9800 Da, average molecular weight was 10500 Da, and both components have biological activity.

2. The medicament of claim 1 wherein the said GMGSP has two characteristic absorption peaks at 195±2nm and 255±2nm wavelength in the UV spectrum.

3. The medicament of claim 1 wherein the said GMGSP is a polypeptide mixture with the molecular weight 5000-12000 Da, which can stimulate DNA synthesis and protein synthesis of primarily cultured myocardial cells, and promote cellular cleavage and proliferation, and protect myocardial cell membrane.

Description

The present invention relates to a growth-stimulating peptide of the myocardial cells (GMGSP). In clinic, GMGSP of this invention can be applied for the treatment of myocarditis disease and the recovery treatment undergoing cardiosurgical operation.

In recent years , the cells that can stimulate DNA synthesis and protein synthesis of myocardial cells, and promote cellular cleavage and proliferation are isolated from the hearts of spontaneously hypertensive rats or experimental hypertensive animals. But their biological activity is not high.

The object of the present invention is to provide a growth-stimulating peptide of the myocardial cells (GMGSP), which was isolated form the hearts of healthy infant mammals. Compared with the recent GMGSP, GMGSP of the present invention has higher biological activity. In clinic, GMGSP of this invention can be applied for the treatment of myocarditis and the recovery treatment undergoing cardiosurgical operation. It can be applied to take orally or intravenous injection with other medicaments.

According to the aspect of the present invention, there is provided a medicament called Lixinkang, which is a stable growth-stimulating peptide of the myocardial cells (GMGSP), wherein the medicament has the followed characteristics:

(1) it is stable at pH 2-9;

(2) its biological activity did not change when GMGSP was heated at 95-100℃ for 10 minutes or at 60-70℃ for 30 minutes;

(3) but its biological activity was lost when being placed in proteolytic enzymes at 37℃ for two hours;

(4) a polymer was formed at 22℃-30℃ in aqueous solution, but its biological activity did not have obvious change;

(5) its biological activity did not change if GMGSP was lyophilized and sealed with

3%-8% mannitol and stored at room temperature for 1.5 years, or at 4℃ for 2 years, or at -20℃ for 3 years;

(6) HPLC analysis indicated that the said GMGSP is composed of four components; and the relative peaks and retention times of each component were respectively 10.4% (2.88 minutes), 6.4% (3.93 minutes), 36.3% (5.09 minutes) and 7.3% (7.41 minutes), and each component has biological activity;

(7) the molecular weights of two bands displayed by SDS-PAGE analysis were respectively 8500 Da and 10800 Da; and the average molecular weight displayed by HPLC analysis was 9800 Da, average molecular weight was 10500 Da, and both components have biological activity.

The cardio myopeptidin of the present invention has two characteristic absorption peaks at 195±2nm and 255±2nm wavelength in the UV spectrum.

Lixinkang of the present invention is a polypeptide mixture with the molecular weight 5000-12000 Da, which can stimulate DNA synthesis and protein synthesis of primarily cultured myocardial cells, and promote cellular cleavage and proliferation, and protect myocardial cell membrane.

HPLC analysis indicated that the said GMGSP is composed of four components. Single component or two components or multiple components in combination are all available.

FIG. 1 is an HPLC analysis of the present invention.

It is indicated from the following experiment that GMGSP of the present invention has higher biological activity. The experiment comprises the steps of: asepsis separating the hearts of 12-16 pregnant SD rats, and isolating the myocardial cells by trypsin, then preparing 2-5X10^5 cells /ml in DME/F12 medium containing 10% calf bovine serum after being washed for 3 times by DMEM medium, then being placed in 96-hole culture plate, each hole contains 0.15ml, then being incubated for 24 hours under the condition of 37 and 5% carbon dioxide. Then the medium was changed into serum-free medium DME/F12, and the experimental holes are added GMGSP ,each hole contains GMGSP 5-40μg, and the control holes are only added

medium DME/F12, then are cultured for another 48 hours, and then each hole is added 5μ IMTT (1.5mg/ml, prepared by DME medium), then are cultured for another 4-6 hours, and each hole is added dimethyl sulfoxide 100μl and the reaction was stopped. And OD value was detected under the enzyme-labelling meter with the length of 570nm. When ratio of the experimental hole/ the control hole is more than 1.7, it has activity. The measured results are as follows:

Effect of GMGSP on mitochondrial dehydrogenase activity in cultured myocytes

	N	OD	Multiples	The value of P
The control group	8	0.108±0.01		
The experimental group (μg)				
5	4	0.143±0.02	1.3	>0.05
10	4	0.233±0.03	2.2	<0.01
20	4	0.243±0.02	2.3	<0.01

TYPE: AREA PERCENT RUN NUMBER: 1 INDEX: 1
WAVELENTH: 254nm

PEAK#	AREA%	RT	AREA	BC
1	0.061	0.28	128537	02
2	0.121	0.98	257530	02
3	1.015	2.69	2150812	02
4	10.356	2.88	21943700	02
5	1.844	3.14	3908476	02
6	1.041	3.35	2205808	02
7	0.874	3.67	1851837	02
8	6.431	3.93	13629270	08
9	1.533	4.51	3248245	06
10	36.337	5.09	76999441	08
11	0.123	5.69	261684	06
12	0.182	6.16	385149	06
13	0.204	6.36	432545	06
14	0.329	6.75	696258	06
15	7.266	7.41	15398112	06
16	2.333	8.29	4942818	06
17	0.667	8.75	1414685	06
18	1.387	9.09	2938040	06
19	0.24	10.19	508311	06
20	1.352	11.15	2864678	06
21	0.804	11.22	1705749	06
22	5.537	12.01	11731315	06
23	3.617	12.13	3426588	06
24	0.348	12.27	737765	06
25	9.506	12.42	20141519	06
26	3.648	15.41	7729768	06
27	2.944	15.53	6238177	06
28	0.632	17.01	1340201	06
29	0.089	17.11	188882	06
30	0.069	17.14	146296	06
31	0.799	17.37	1692180	06
32	0.311	19.66	659510	07
TATAL	100		211906886	

The pharmacological effect is carried out by the following technique:

SD rats (male or female) were selected and were injected at a dose of 2mg/kg body weight with isoproterenol. Then the rats were treated by GMGSP after 24 hours, each rat was administered GMGSP 4mg, one time per day lasting for 3 days. GMGSP was replaced by normal saline in control group, and the SD rats were killed after 3 days. Then performed the pathological section of the heart and observed the differences of lesions between the treat group and the control group.

Results:

The pathological change of the control group mainly manifests myocardium showed degeneration (mainly the blister changes of myocardium). Serious necrosis is observed around the arteriolar, and the cross striation of myocardium is shallowed. There has a fracture phenomenon among the myocardial cells. The extent of lymphocytic infiltration is obvious. But the GMGSP group, the extent of myocardium showed degeneration and necrosis is lighter compared with the control group, and the number of myocardial necrosis is few, and the extent of lymphocytic infiltration is light, and the myocardial structure of cardiomyopathy is clear.

The medicament of this invention can be used singly, and also can be used in combination with the Chinese and western simple prescription or combine compound which can promote myocardial metabolism and repair myocardial injury and improve myocardial blood circulation.

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[56]参考文献

CN1033564	1989. 7. 5	A61K35/78
CN1061156	1992. 5.20	A61K35/12
CN1081323	1994. 2. 2	A23C9/152

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权利要求书 1 页 说明书 4 页 附图页数 1 页

[54]发明名称 利心康

[57]摘要

本发明涉及的是一种治疗心肌疾病的药物—利心康，它是从健康幼年哺乳动 物如乳猪、牛等心脏提取的心肌细胞生长刺激肽（CMGSP），分子量为 5 000—12000Da，它实际上是一种多肽混合物，生物活性稳定，能刺 激原代培养心肌细胞 DNA 及蛋白质的合成，促进心肌细胞分裂增殖及保护心 肌细胞膜，临床应用时可采用口服和肌肉注射，也可与其它药物配伍静脉滴入 ，在治疗心肌疾病方面显示出很好的效果。

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权 利 要 求 书

1.一种心肌炎治疗药，心肌细胞生长刺激肽(GMGSP)，是从健康幼年哺乳类动物心肌中制备提取，其特征是：

(1)在 pH2—9 范围内稳定；

(2)加热 95—100℃ 10 分钟，60—70℃ 30 分钟条件下生物活性不改变；

(3)在多种蛋白水解酶，37℃ 2 小时条件下生物活性丧失；

(4)在水溶液 22—30℃ 条件下可形成聚合体但生物活性改变不明显；

(5)在加入 3—8% 甘露醇冻干密封条件下，室温贮存 1.5 年，4℃ 贮存 2 年，－20℃ 贮存 3 年，生物活性不改变；

(6)在 HPLC 分析条件下，由 4 个组份组成，各组份相对峰面积为：峰 1　10.4%，峰 2　6.4%，峰 3　36.3%，峰 4　7.3%，各峰保留时间分别为峰 1　2.88 分，峰 2　3.93 分，峰 3　5.09 分，峰 4　7.41 分。每个组分单独实验，均有生物活性；

(7)经 SDS—PAGE 分析，显示 2 条带，其分子量分别为 8500Da，10800Da，经 HPLC 分析，GMGSP 的数均分子量为 9800Da，重均分子量为 10500Da，2 个组分均有生物活性。

2.根据权利要求 1 所述的心肌炎治疗药，其中心肌细胞生长刺激肽紫外扫描光谱图为，在 195±2nm 及 255±2nm 处有 2 个特征性的吸收峰。

3.根据权利要求 1 所述心肌炎治疗药，其中心肌细胞生长刺激肽是能刺激原化培养的心肌细胞的 DNA 合成和蛋白质合成，促进心肌细胞的分裂增殖及保护心肌细胞膜的分子量为 5000—12000Da 的小分子多肽混合物。

说　明　书

利　心　康

本发明涉及的是一种心肌细胞生长刺激肽(CMGSP)，临床应用于心肌炎和心脏外科手术后的恢复治疗。

现有技术中具有刺激心肌细胞DNA和蛋白的合成，促进细胞分裂增殖的刺激因子，主要是从自发性高血压大鼠或实验性高血压动物的心肌中提取，生物活性较低。

本发明的目的在于提供一种从健康幼年哺乳类动物的心肌中提取出的具有较高生物活性的心肌细胞生长刺激肽(CMGSP)，供临床上用于治疗心肌疾病和心脏外科手术后的恢复治疗。可口服和肌肉注射，或与其它药物配伍静脉。

本发明利心康药物是一种稳定的心肌细胞生长刺激肽(CMGSP)，具有如下特征：

(1)在PH2--9范围内稳定；

(2)加热95--100℃10分钟，60--70℃30分钟条件下生物活性不改变；

(3)在多种蛋白水解酶，37℃，2小时条件下生物活性丧失；

(4)在水溶液22--30℃条件下可形成聚合体，但生物活性改变不明显；

(5)在加入3--8%甘露醇冻干密封条件下，室温贮存1.5年，4℃贮存2年，-20℃贮存3年，生物活性不改变；

(6)在HPLC分析条件下，由4个组份组成，各组份相对峰面积为：峰1　10.4%，峰2　6.4%，峰3　36.3%，峰4　7.3%，各峰保留时间分别为峰1　2.88分，峰2　3.93分，峰3　5.09分，峰4　7.41分。每个组份单独实验，均有生物活性；

(7)经SDS--PAGE分析，显示2条带，其分子量分别为8500Da，10800Da，经HPLC分析，CMGSP的数均分子量为9800Da，

重均分子量为10500Da，2个组份均有生物活性。

经紫外扫描光谱图为，在195±2nm及255±2nm处有两个特征性的吸收峰。

利心康是为一类具有刺激原代培养的心肌细胞的DNA合成和蛋白质合成，促进心肌细胞的分裂增殖及保护心肌细胞膜的分子量为5000--12000Da的小分子多肽混合物。

其分成四个组份，单一组份或二种及多组份组合使用均有效。

附图1为本发明HPLC分析

本发明药物生物活性表现方法实例：

取SD胚胎鼠（孕12--16天），无菌分离心脏，胰蛋白酶分离心肌细胞，用DMEM培养基洗涤3次后，用含10%小牛血清的DME/F12培养基配制2-5×10^5个细胞/ml，置96孔培养板内，每孔0.15ml，37℃，5% CO2条件下孵育24小时。用无血清DME/F12培养基换液后，实验孔加入5-40μg/孔的CMGSP，对照孔仅加DME/F12培养基，继续培养48小时，每孔加入5μlMTT(1.5mg/ml，DME培养基配制)，继续培养4--6小时，每孔加入二甲基亚砜100μl终止反应置酶标仪570nm条件下测OD值，以实验孔/对照孔>1.7倍为有活性。测定结果如下：

CMGSP对培养中心肌细胞线粒体脱氢酶活力的影响

	n	OD	倍数	P值
对照组	8	0.108±0.01		
实验组（μg）				
5	4	0.143±0.02	1.3	>0.05
10	4	0.233±0.03	2.2	<0.01
20	4	0.243±0.02	2.3	<0.01
40	4	0.195±0.04	1.8	<0.05

本发明药物的药理作用实现如下：

取SD大鼠，雌雄不限，每kg体重注射异丙肾上腺素2mg，24小时后，用CMGSP治疗，每只大鼠给CMGSP 4mg，每日一次，连续三天。用生理盐水作对照，3天后杀鼠，取心脏做

TYPE: AREA PERCENT RUN NUMBER: 1 INDEX: 1

WAVELENGTH: 254nm

PEAK#		AREA%	RT	AREA	BC
1		0.061	0.28	128537	02
2		0.121	0.98	257530	02
3		1.015	2.69	2150812	02
4	①	10.356	2.88	21943700	02
5		1.844	3.14	3908476	02
6		1.041	3.35	2205808	02
7		0.874	3.67	1851837	02
8	②	6.431	3.93	13629270	08
9		1.533	4.51	3248245	06
10	③	36.337	5.09	76999441	08
11		0.123	5.69	261684	06
12		0.182	6.16	385149	06
13		0.204	6.36	432545	06
14		0.329	6.75	696258	06
15	④	7.266	7.41	15398112	06
16		2.333	8.29	4942818	06
17		0.667	8.75	1414685	06
18		1.387	9.09	2938040	06
19		0.24	10.19	508311	06
20		1.352	11.15	2864678	06
21		0.804	11.22	1705749	06
22		5.537	12.01	11731315	06
23		3.617	12.13	3426588	06
24		0.348	12.27	737765	06
25		9.506	12.42	20144519	06
26	⑤	3.648	15.41	7729768	06
27		2.944	15.53	6238177	06
28		0.632	17.01	1340201	06
29		0.089	17.11	188882	06
30		0.069	17.14	146296	06
31		0.799	17.37	1692180	06
32		0.311	19.66	659510	07
TOTAL		100		211906886	

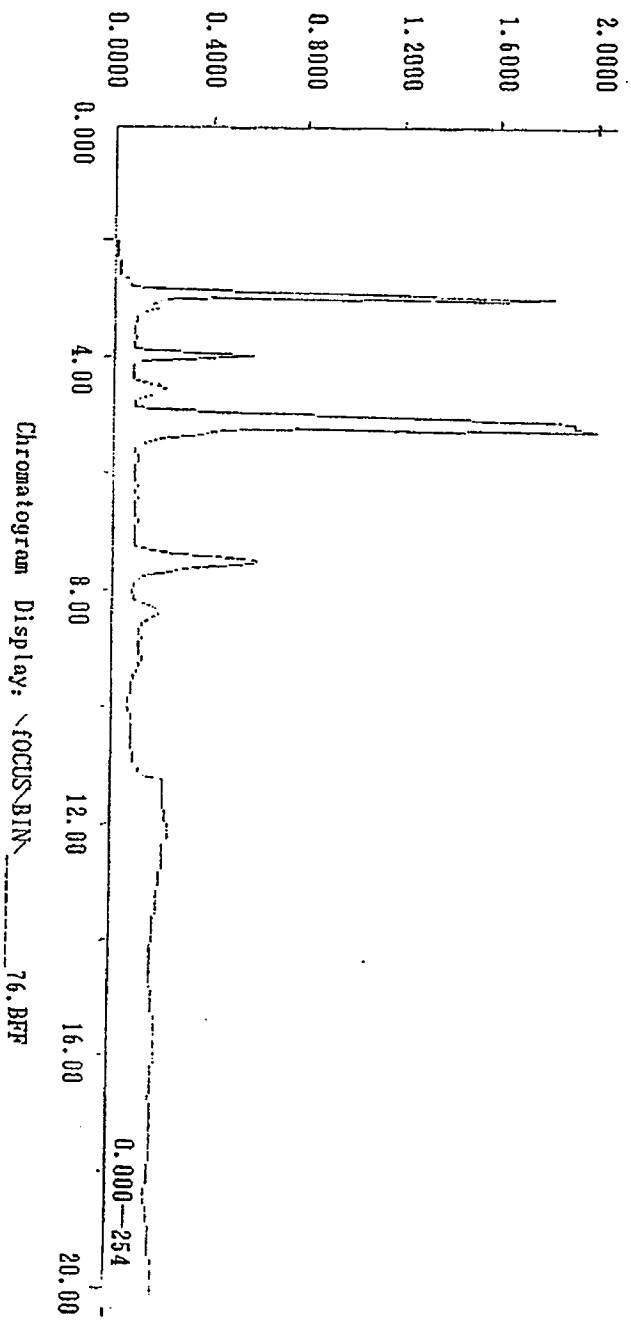
病理切片，观察治疗组与对照组的病变差异。

结果发现：

对照组的病理改变主要表现为心肌细胞变性（水泡样变为主），严重的坏死多见小A周围，心肌横纹变浅，心肌细胞之间有断裂现象，淋巴细胞浸润程度明显。而CMGSP组心肌细胞变性坏死的程度均较对照组为轻，坏死细胞的数量也较少，淋巴细胞浸润程度轻，心肌细胞结构清晰。

本发明药物可单独使用，亦可与其它促进心肌代谢、修复心肌损伤，改善心肌血液循环的中西药单方或复方合用。

说 明 书 附 图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